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A MAILING-CASE METHOD FOR LONG-DISTANCE BACTERIOLOGICAL CONTROL OF WATER SUPPLIES¹

BY M. H. McCrady²

The absolute necessity of laboratory control of water purification processes needs no argument. The general recognition of such necessity is exemplified in the universal practice of installing a local laboratory in every large purification plant, and in the more recent attempt by some State Departments of Health to train local plant operators to make their own laboratory tests. Probably every State and Provincial Department makes an effort to determine, at more or less regular intervals, the efficiency obtained by the plants under their supervision.

The most generally adopted feature of this laboratory control is the examination of bacteriological samples. Such examinations must be frequent, if they are to answer their purpose effectively. The frequent variations in the quality of the raw water, the limitations of mechanical equipment, and the uncertainty of the human element, all create a demand for as nearly continuous laboratory supervision as economy will permit.

In plants with local laboratory service, the ideal of frequency of examination may be closely approached. Samples may be taken daily or many times a day, as conditions may demand. But for the small plant, without local laboratory service, in charge of a man to whom a purification apparatus often means simply increased labor and responsibility without extra remuneration, the duty of maintaining effective laboratory control is passed on to the State or Provincial Department of Health, and there the problem assumes a real magnitude.

It is needless to dwell upon the difficulties encountered in long-distance bacteriological control of water supplies, the bulky and

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expensive equipment required, the time consumed in preparing this equipment for shipment, the cost of shipment, the demand for co-operation on the part of the local man who is to collect and ice the samples, and last, but not least, the expenditure of time and labor in analyzing the samples thus obtained. These difficulties are a common experience to water laboratory men, and their reality is indicated by the infrequency with which samples are usually obtained from plants situated at some distance from the central laboratory.

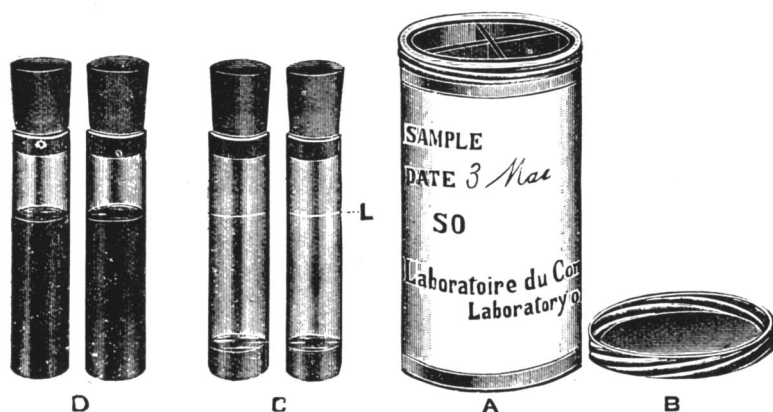


FIG. 1. DAILY SAMPLING OUTFIT FOR LONG-DISTANCE CONTROL OF WATER SUPPLIES

The mailing-case (A) with screw top (B) fitted with a cardboard partition to hold the four glass tubes.

(C) shows two of the tubes as sent out by the Laboratory. Each contains 2 cc. of concentrated lactose medium.

(D) shows two of the tubes as returned to the Laboratory. The municipal officer has added water up to the mark (L) on the tube from the municipal supply to be tested.

After the water is added to the four tubes as shown in (D), the tubes are replaced in the mailing-case, the date of sampling marked on the case, and the case is mailed to the Laboratory, three cents postage being required.

In the effort to avoid some of these difficulties, it occurred to the author that, by sacrificing to a certain extent, completeness of examination to greater frequency of examination, a method of sampling and analysis might be evolved which would permit of daily or even more frequent sampling, and yet be comparatively inexpensive and demand very little time at either the point of collection or the laboratory.

The object of this paper is to describe briefly such a method, which has been employed for two years by the Laboratory of the Superior Board of Health of the Province of Quebec.

The principle of this method is the daily shipment of samples of water for the determination of the presence of *B. coli*. The details of the method and apparatus are given at the end of the paper, but the procedure may be indicated as follows: Lots of 20 or 30 small mailing cases are shipped by the laboratory to the town having the water supply to be examined. Each mailing case contains four cork-stoppered glass tubes, graduated at 7 cc., and containing 2 cc. of concentrated medium. Every day the four tubes of one case are filled to the mark by the sample collector with the water to be tested, each tube thus containing 5 cc. of sample, and the case is mailed to the laboratory. At the laboratory a small inverted vial is placed in each tube, the tube tipped in order to fill this vial, and the whole then incubated at 37°C. in the usual manner, results being noted after 24 and 48 hours' incubation. Further identification, in case of positive results, may be practised if required.

Certain features of this mailing method may be noted:

The outfits are inexpensive, for the cases, tubes, vials, etc., are all of standard size, and are readily prepared for use by laboratory assistants.

Time of transit is reduced to a minimum, for the excellent service of the mails is utilized.

Cost of shipment is extremely small, for the case goes through the Canadian mail for the insignificant postage cost of three cents, under the classification of "sample."

The very little labor entailed in the collection of samples ensures a surprising regularity in such collection, and renders it possible to enlist the services of any physician or municipal officer for this purpose.

Check samples from the same town may be multiplied.

At the laboratory a large number of cases may be received each day, for they may be handled at the rate of about one per minute.

A day is often gained in obtaining results by this method as compared with ordinary bottle samples, for, because of multiplication of the organisms during transit, ultimately positive tubes are usually positive after one day of incubation.

Each day's result is quantitative, for the case contains four samples of 5 cc. each, and the total of 20 cc. permits a fairly complete test for *B. coli*.

Breakage in the mails is an insignificant item, and even when, as has happened once or twice, four tubes were broken, there was no evidence of leakage or stain on the outside of the case, for the liquid is readily absorbed by the paper packing.

Overgrowth of *B. coli* by other organisms in transit does not appear to be an important factor. Occasionally a case has been received which had been held four or five days, and yet fermentation of an apparently normal character proceeded upon incubation. So far as it has been possible to judge from many control bottle samples and from two or three more extensive comparisons in the laboratory, this mailing method appears to be quite trustworthy, and although results from it may not be so satisfactory or complete as those obtained from bottle samples collected in the usual manner, its inexpensiveness and general applicability have demonstrated its utility.

In the Province of Quebec this system of sampling has been employed for a variety of purposes, but particularly in long-distance control of purification plants it has been of the greatest service. For it is the practice to post, every day, in a chart in the office of the sanitary engineer who is charged with the supervision of these plants, the results obtained. Possession of such a daily record permits concentration of attention on those plants which are really in need of improvement, and in the case of isolated unsatisfactory results, a telephone communication followed, if necessary, by a personal visit to the plant in question, serves not only to frequently discover failures in the mechanical equipment, but also to maintain a lively interest on the part of the operator, an interest further stimulated by receipt of a weekly statement of the results obtained.

To date, the laboratory for this Province has examined over 18,000 tubes of water sent through the mails. These samples have, in the main, come from 15 or 20 different purification plants. Now that further supplies of mailing cases are obtainable, it is intended to extend this service to other plants in the Province.

Two years of experience with this method of sampling and analysis have proved it to be extremely simple and practicable, and the author believes that it supplements, effectively and satisfactorily, the more infrequent examination of the bottle samples usually employed for long-distance bacteriological control of water supplies.

DETAILS OF EQUIPMENT AND MANIPULATION

Mailing case. Metal can, lined with paper board, with screw top.

Outside diameter, $2\frac{1}{4}$ inches; length, $3\frac{3}{4}$ inches; supplied by Improved Mailing Case Company, New York. Extra paper inserts in cover and bottom to absorb liquid in case of breakage.

Partition of double-faced corrugated straw-board to divide the can into four compartments. The paper-box manufacturer who supplies this board cuts it into rectangles $2 \times 3\frac{1}{2}$ inches, and laboratory assistants cut and fit the rectangles to make the partitions.

Cans and covers are sterilized before use by dry heat in the usual manner.

Sample tubes. Specimen tubes (shell-vials) flat bottom, straight wall, outside diameter, $\frac{5}{8}$ -inch; length, $2\frac{7}{8}$ inches, graduated with a mark at 7 cc. This is done with a pipette, the meniscus marked with a wax pencil, and with a small file wet with water, a cut of about $\frac{1}{4}$ inch in length is made in the glass at the pencil mark. The tube will not break at this mark upon sterilization.

Concentrated medium. This medium may be any that the laboratory may elect, but so constituted that the 2 cc. of this medium, when added to 5 cc. of water sample, will give the required concentration of mix.

Preparation of tubes and cases. The tubes are placed in a zinc rack holding about 48 tubes, and 2 cc. of the concentrated medium is placed, by means of a pipette, in each tube. First-class cork stoppers are then fitted into the tubes, rather loosely to avoid their blowing out upon sterilization. Corks are of the long form, which, when thrust home, will project about $\frac{2}{3}$ inch beyond the tube. A deep zinc box cover is then placed over the rack to keep the corks in the tubes by its weight and to protect the tubes from undue contamination, and the whole then sterilized in the autoclave.

After sterilization, the cover being removed, each cork is firmly thrust into its tube, and the tube placed in its compartment in the mailing case. When the case contains its four tubes, the cover is screwed on, and the label affixed to the body of the case. This label is printed with the address of the laboratory, the word "Sample" to place it in the proper postal classification, and the word "Date" after which the sample-collector is to write the date of collection of samples.

When shipping a lot of cases to a town, a number or group of letters for that town is stamped on the label in order to identify the case upon its return to the laboratory.

Collection of samples. The sample-collector, with usual precautions, fills each of the four tubes of a case with water to the mark on the tube, writes the date of sampling on the label, and affixes a three cent stamp.

Laboratory procedure. Upon receipt of the case at the laboratory, it is identified by the number or group of letters on the label, and the date of collection and of receipt noted on the record sheet. Each tube is removed from the case, its cork stopper withdrawn, and a small vial or specimen tube, having an outside diameter of $\frac{1}{4}$ inch and length of 2 inches, is inserted, inverted, into the tube. (These vials are contained in a beaker, covered with a Petri dish, the whole having been previously sterilized. The vials are taken from the beaker with flamed tweezers.) The cork is then replaced, the tube tipped so that the small vial is filled with the liquid in the tube, and the tube then placed in a wooden rack, which consists of a block 12 x 3 x 1 inch thick, containing four groups of four holes each. The four tubes from the case go into the holes of one of these groups, and the day of collection and day of receipt are written in pencil on the wood near this group of tubes. Each town from which samples are received has its own rack, permanently labelled. The rack is then placed in the 37°C. incubator.

Reading of results. Results are read after 24 and again after 48 hours. At least 10 per cent of gas is required for a positive result, as it is difficult to remove every last small bubble from the vial when the latter is inserted into the tube.

Laboratory record. The record form used by this laboratory is the following:

June, 1920							
SHERBROOKE				SOREL			
1	2	1	2	1	2	0	0
2	4	0	0	2	3	1	1

From the above record, a case received from Sherbrooke on June 2, samples having been collected on June 1, had 1 tube positive after 24 hours incubation, and 2 tubes positive after 48 hours incu-

bation. Case sent on June 2, and received on June 4 had no tubes positive after either 24 or 48 hours incubation.

Range of results. The range of numbers of *B. coli* corresponding to results obtained with four, three, etc., tubes of 5 cc. of sample each, is shown below (as probable numbers of *B. coli*):

Numbers of B. coli corresponding to different results with one to four sample tubes

4 TUBES		3 TUBES		2 TUBES		1 TUBE	
Positives	B. coli per 100 cc.	Positives	B. coli per 100 cc.	Positives	B. coli per 100 cc.	Positives	B. coli per 100 cc.
0/4	0	0/3	0	0/2	0	0/1	0
1/4	6	1/3	8	1/2	14	1/1	14+
2/4	14	2/3	22	2/2	22+		
3/4	28	3/3	†28+				
4/4	*32+						

* Considered as a 4/5 result, at least.

† Considered as a 3/4 result, at least.